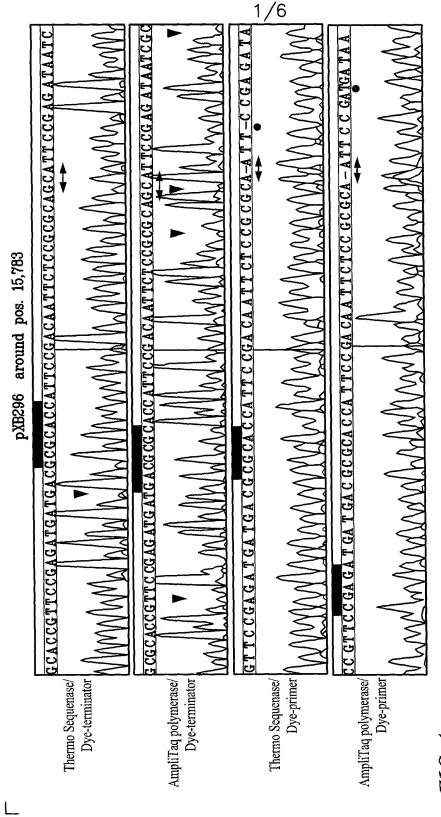
Sheet 1 of 6 Application Serial No.: 09/939,964 Title: Genomic Sequence of NGR 234 Symbiotic Plasmid, Its Gene Map, And Its Use In Diagnostics And Gene Transfer In Agriculture Inventors: Andre Rosenthal, Christoph Bernward Freiberg, Xavier Philippe Perret and William John Broughton
Docket No. CARP-0094
Atty: Doreen Yatko Trujillo

Telephone No. (215) 568-3100

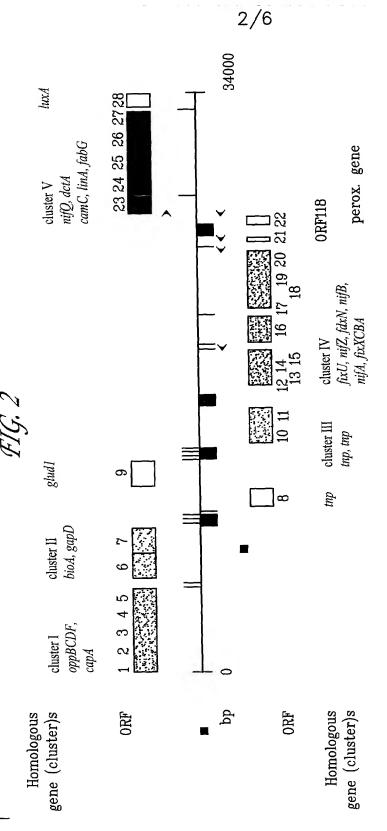


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position 15,783 (verticle line in the middle of each sequence). The readings cover a part of the minus strand of pXB296. (🔻) Extremely low electropherograms (traces) with the corresponding sequences generated by automatic base calling are shown around the pXB296 sequence signals produced with dye terminators; (->) the sequence GCA, which is compressed in dye primer scans; (-) automatic base-calling Comparison of sequences from pXB296 created by different cycle sequencing methods. The graphic outputs (program XGAP) of four inaccuracies.

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Atty: Doreen Yatko Trujillo

Diagnostic August 27, 2001
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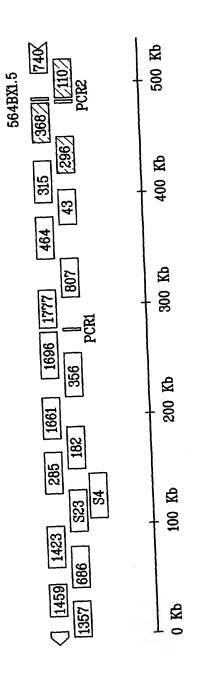


arrowhead), similar to $\vec{\sigma}^{54}$ -dependent promoter consensus sequences (TGGCACG-N₄-TTCG) + nifA upstream activator clusters (\blacksquare), which might function as ρ -independant transcription terminators, are represented. Sequence motifs (open sequences (TGT-N $_{10}$ -ACA), are located at the following positions on the cosmid: 19,107 - 19,120 + 19,195-19,210; 24,787 - 24,800 + 24,878 - 24,893; 25,508 - 25,521 + 25,567 - 25,582; 26,815 - 26,828 + 26,941 - 26,956 (all minus algorithms) (algorithms) (algorOrganization of the predicted ORFs in pXB296 from Rhizobium sp. NGR234. Significant stem-loops (1)/stem-loop strand); 27,074 - 27,131 (several possibilities) + 26,969 - 26,984(plus strand)

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564BX1.5 and PCR2: a subcloned 1.5-Kb DNA-fragment derived from cosmid pXB564 and a PCR product covering the gap Map of the 20 sequenced cosmids covering the 536-Kb symbiotic plasmid of Rhizobium sp. NGR234. The cosmid names consist of the prefix pXB and numbers that are given in the graphic. PCR1:PCR product covering the gap between two cosmids from position 276,448 to 277,944.

Grey coloured areas cover the region from pos 417,796 to 517,279 mentioned in the text. between two cosmids from pos. 408,607 to 483,991.

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Sheet 4 of 6
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		10	20 _	
pRiA4b	CGCAAAA	AAAAGA	GCCCCCTC	A A C G T C G C C A C G G T A A C C A A C G G C G
pNGR234a	CGCAAAAC	G A A A A A G (G C C C C C A G	AIC GIGIT AIA CIC
pTiB6S3	CGCAAAA	G A A A A A G G	G C C C C C G A	A A C GIG TIGIE G
pRL8JI	CGCAAA	GAAAAA G	CCCTCCGA	A A C G G I G - G
		40	50	60 C - T C T A G C - C G T T T A G C A A G T C T C G C A
pRiA4b	GT CGC G G	A GCC CT	TCTGTC-T	C-TCTAGC-
pNGR234a	AT CGTIGG	A A GICICI	TCTCTCAT	CGTTTAGCA
pTiB6S3	TT C-CGG	A A G A C C T	TCTCT-AT	AGTICITICIGICIA
pRL8JI	TTC-CAG	A A GIGIC CI-L	TCTCTCA-	-GTTTGGT C
•		7 0	80	90
pRiA4b	G C G A A C A	GAATCGC	ATTTCCTC	GAATCCTCG GAATCCCAG GAATCCCAA
pNGR234a	GCCTG-A	GAATCGC	ATTTCCAC	GAATCGCAG
pTiB6S3	G CT AIAIG A	GAATCGC	ATTTTCAG	GAATCCCAG
	COTTACA	CAATCCC	ATTTCCCCG	GAATCACAG
DUTOTI	IG CIL LIAIGIA	GRAICGO	A I I I O O O	
pRL8JI)	110	120
•	100)	110	120
pRiA4b	100 T C A A G A G)	110	120
pRiA4b pNGR234a	100 T C A A G A G T C A A G A G)	110	120
pRiA4b pNGR234a pTiB6S3	100 T C A A G A G T C A A G A G T C A A G A G) TT T — — TTT T C T — — TT T C C C G T G	110	120
pRiA4b pNGR234a	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G) T T T T T T C T T T T C C C G T G T C	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T -
pRiA4b pNGR234a pTiB6S3 pRL8JI	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G) TTTTTTT TCCCCGTG TC	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G
pRiA4b pNGR234a pTiB6S3 pRI&JI pRiA4b	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G) TTTTTTT TCCCCGTG TC	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G
pRiA4b pNGR234a pTiB6S3 pRL8JI pRiA4b pNGR234a	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C G A G C G A C G A G C		110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G
pRiA4b pNGR234a pTiB6S3 pRI&JI pRiA4b	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G		110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T -
pRiA4b pNGR234a pTiB6S3 pRIAJI pRiA4b pNGR234a pTiB6S3	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C C A G C C A C G A G C C A C G A G C C A C G A G C		110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G
pRiA4b pNGR234a pTiB6S3 pRL8JI pRiA4b pNGR234a pTiB6S3 pRL8JI	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C C A G C		110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G
pRiA4b pNGR234a pTiB6S3 pRI&JI pRiA4b pNGR234a pTiB6S3 pRI&JI	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C C A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C	TTTT-TTTTTCGGATTCCTTCCTTCCTTCCTTCCTTCCTTCCTTC	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G
pRiA4b pNGR234a pTiB6S3 pRL8JI pRiA4b pNGR234a pTiB6S3 pRL8JI pRiA4b	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C C A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C	TTTTTTTTCCT-TTTCCT-TTTCCTTTTCCTTTTCCTTTTCCTTTTCCTTTTCCTTTTCCTTCTTTT	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G
pRiA4b pNGR234a pTiB6S3 pRI&JI pRiA4b pNGR234a pTiB6S3 pRI&JI	100 T C A A G A G T C A A G A G T C A A G A G T C A A G A G T C A A G A G C A G C C A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C C A C G A G C	TTT T - TTT T C C C G T G T C T G A T T T C G G A C G T C T G A T T T C C T - T T T C C T - T T T C	110 A G C G C C G T G G C A C C G G A G G A A A G T A A C G C C A C 140 C T T T G C C T T T T G C C T	120 T T T G G T - A A C G G - G T - A T C G T - T T C A C C G G C G T - 150 G C T G A A A G G G A G G A T A G G A A C - A A A G G

Multiple alignments of the nucleotide sequence of the replication origins of: the Ri plasmid of Agrobacterium rhizogenes (pRiA4b), the symbiotic replicon of NGR234 (pNGR234a), the Ti plasmid of A. tumefaciens BS63 (pTiBS63) and pRL8JI of R. leguminosarum bv. leguminosarum (pRL8JI). Gaps introduced to give the best sequence alignments are marked by hyphens.

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Atty: Doreen Yatko Trujillo
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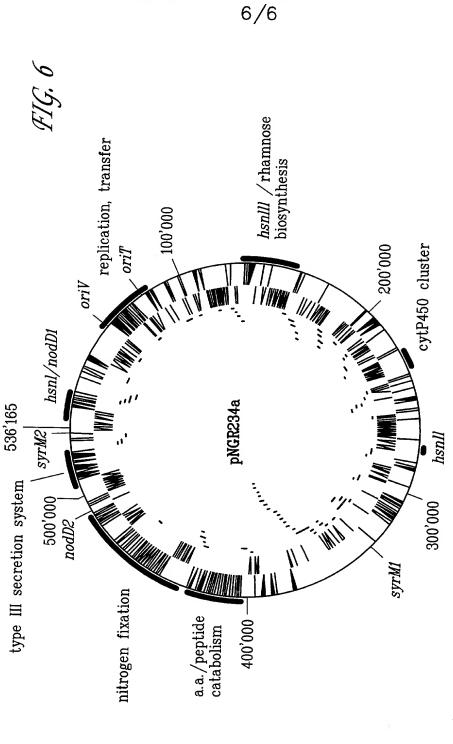
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specific plasmid strand is cleaved are marked (z). Sequence features in the trailing portion of the oriT sites include inverted repeats which Multiple DNA sequence alignments of the regions containing the origin of transfer of: the symbiotic plasmid of NGR234; pTiC58, the Ti ferrooxidans. Major conserved nucleotide residues are boxed. Known "nick" sites corresponding to the nucleotide positions where the plasmid of A. tumefaciens C58, RSF1010, a mobilisable plasmid of E. coli, and, pTF1, a mobilizable plasmid of Thiobacillus are marked by horitzontal arrows.

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Sheet 6 of 6
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Circular representation of the symbiotic plasmid of NGR234. Outer and inner concentric circles: coding regions identified on the plus and minus strands respectively. ORFs which belong to insertion-like elements are not visible. Thin concentric black lines represent mosaic sequences as well as complete or partial IS like repeats. Major gene clusters are highlighted as thick black concentric lines.